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The Embryo–Fetal Toxicity and Teratogenic Potential of Ammonium Perfluorooctanoate (APFO) in the Rat

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The Embryo–Fetal Toxicity and Teratogenic Potential of Ammonium Perfluorooctanoate (APFO) in the Rat. STAPLES, R. E., BURGESS, B. A., AND KERNS, W. D. (1984). *Fundam. Appl. Toxicol.* 4, 429–440. Ammonium perfluorooctanoate (APFO, >95% pure) was administered to Sprague–Dawley rats from Days 6 through 15 of gestation by inhalation as a dust (whole body exposure) for 6 hr/day at 0, 0.1, 1, 10, and 25 mg/m^3, or by gavage at 100 mg/kg body wt/day in corn oil. Maternal deaths occurred in the groups given the highest level of APFO by each route and overt toxicity was evident among the surviving dams of these groups and among those of the 10-mg/m^3 group. The fetuses were examined for external, visceral, and skeletal alterations and for APFO-related macroscopic and microscopic alterations of the eyes. In the postpartum period, pups from additional control and experimental dams were examined externally and ophthalmoscopically, and the usual fertility and viability indices were calculated. A teratogenic response was not demonstrated. Toxic effects on the conceptus were noted only in the groups given the highest level of APFO by each route. Hence, APFO was not demonstrated to represent a unique hazard to the conceptus of the rat.

Ammonium perfluorooctanoate (APFO)[2] is a representative of an important class of compounds, the perfluorocarboxylic acids and their salts. Their teratogenic properties have not been studied extensively. Its toxicity in several animal species was reported by Griffith and Long (1980). The oral LD50 in the rat was 540 mg/kg with the liver being the most sensitive target. In the rhesus monkey the gastrointestinal tract and the reticuloendothelial system were the sites of toxic effects after dietary exposure. By inhalation, the approximate lethal dose (ALD) in the male rat after a 4-hr, head only, exposure was 80 mg/m^3 (unpublished Du Pont data).

Du Pont purchases APFO for use in the manufacture of a variety of fluoropolymer resins, dispersions, and elastomers. Between the last part of 1980 and March 1981, the manufacturer reported to the U.S. Environmental Protection Agency (EPA) under Section 8(e) of the Toxic Substances Control Act (TSCA)[3] that APFO and several related chemicals[4] had demonstrated teratogenic activity in rats. The reported teratogenic activity consisted of lens changes in the eyes of near-term offspring of rats exposed to the test chemicals by gavage from Days 6 through 15 of gestation. The changes included macroscopically evident discoloration of the fetal nucleus of the lens, apparent abnormal arrangement of lens cells to a varying degree, and clefts in the anterior portion of the lens. Significant dose responses

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[2] Octanoic acid, pentadecafluoro-, ammonium salt; ammonium perfluorooctanoate; ammonium perfluorocaprylate; CAS Registry No. 3825-26-1.

[3] Section 8(e) Nos. 373 and 374 on November 19, 1980; Section 8(e) No. 394 on March 20, 1981.

[4] Potassium salt of perfluoroalkyl sulfonates; *N*-ethyl perfluorooctyl sulfonamido ethanol; *N*-ethyl perfluoroheptyl sulfonamido ethanol.

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were reported with incidences of up to 100%, with 0% incidence in the control groups.[3] It was not determined whether the lens changes persisted in fetuses born and raised to weaning age.

As a precautionary measure, female employees in both companies were removed from exposure to APFO and additional teratogenicity testing was initiated in animals. At Haskell Laboratory (Du Pont) the inhalation route was tested to determine whether the reported teratogenicity of APFO in the rat would be expressed after exposure by this route and, if so, to establish an apparent "no-effect" concentration for protection of the conceptus. The information gained was to aid in establishment of workplace standards for women of childbearing potential. Rats also were given APFO by gavage to confirm or refute the preliminary findings reported to the EPA, and if confirmed, to determine whether the changes observed persist after birth.

METHODS

Test material. The APFO sample [$CF_3(CF_2)_6COONH_4$] was obtained from Du Pont's supply previously purchased from the manufacturer.[5] Its purity was >95%; the contaminants present were [$CF_3(CF_2)_4COONH_4$] and APFO isomers. No inhibitors, carriers, or additives were present. Degradation of APFO is insignificant unless temperature exceeds 250°C (unpublished Du Pont data).

Generation and sampling of concentrations of APFO. Dust atmospheres of APFO were generated with an airtight, two-stage glass apparatus composed of a round bottom reservoir and a cyclone-shaped elutriator. A generation air stream introduced at the bottom of the reservoir carried dust particles upward to the elutriator. Dilution/carrier air entered tangentially into the top of the elutriator and swept airborne dust particles into the exposure chamber.

To determine chamber concentrations of APFO gravimetric samples were taken from all chambers at regular intervals (low at 1 hr; intermediate and high at 0.5 hr). A known volume of chamber air was drawn through preweighed Gelman glass fiber filters (Type AE, 25 mm). The filters were reweighed and APFO concentration was calculated from the gain in filter weight. As a back-up system the APFO collected on the filters was extracted and analyzed spectrophotometrically (Percival, 1968). All samples taken from the low concentration were analyzed by this procedure, as were five or six samples per exposure period from the intermediate and high concentrations. Filter samples taken from the control chamber also were analyzed periodically.

Chamber temperatures were monitored each hour. Particle size at the high concentration was determined during Trials I and II through use of an eight-stage Sierra Cascade Impactor Model 218K.

Administration of APFO by gavage. In the preliminary study conducted by the manufacturer the APFO was suspended in corn oil and given to pregnant rats by gavage. Hence, for the current study stripped corn oil was used.[6] During the dosing period (Days 6–15 of gestation), suspensions were prepared daily in corn oil such that 100 mg APFO/kg body wt was contained in 5 ml of suspension/kg body wt. The body weight most recently recorded was used to calculate the dose to be given to each dam. A sample of the suspension remaining after completion of each day's dosing was stored at about 4°C to be available for analysis of concentration and of uniformity of mixture. The control group in each experiment received 5 ml stripped corn oil/kg body wt for the same period of gestation.

Corn oil suspensions of APFO were analyzed for fluorine content by decomposition in an oxyhydrogen flame using the standard Wickbold apparatus (Bock, 1979). The fluoride ion content in the condensed vapors was measured by thorium nitrate titration (Williams, 1979). A standard sample of *m*-carboxybenzotrifluoride was burned and titrated with each batch to verify accuracy of the analytical method for fluoride.

Animals. At arrival, nulliparous, female rats[7] (Sprague–Dawley derived Crl:CD (SD)BR strain) weighed between 151 and 198 g and were about 55 days of age. Male rats of the same strain from the same source ranged from the same age as the females to 1 month older. The rats were conditioned to the laboratory environment for a minimum of 10 days. A standard laboratory diet[8] and water from the Wilmington Suburban Water Corporation were supplied *ad libitum*. The animal rooms were lighted from 6:00 AM to 6:00 PM daily, and were maintained between 22 and 25°C, with 36–70% relative humidity (the 95% confidence interval was 50.3–52.0% in the AM and 48.8–50.4% in the PM).

Since cataracts or opacities occur among adult CD rats, all prospective parental rats were examined for these alterations before breeding. The eyes of each rat were dilated

[5] 3M, 3M Center, St. Paul, Minn. 55144.

[6] CAS Registry No. 8001-30-7; Item 13266, Lot D4-45, Eastman Kodak Company, Rochester, NY.

[7] Charles River Breeding Laboratories, Inc., North Wilmington, Mass. 01887.

[8] Purina Certified Rodent Chow 5002, Checkers, Ralston Purina Co., Checkerboard Square, St. Louis, Mo. 63188.

with 1% atropine ophthalmic solution[9] and examined in semidarkness by a consultant ophthalmologist[10] using focal illumination, indirect ophthalmoscopy, and, when indicated, slitlamp microscopy. Rats with eye lesions were eliminated from the colony before the breeding began.

Experimental design and procedures. For the inhalation route (Table 1) the concentrations of APFO selected for study were 0, 0.1, 1, and 25 mg/m^3. The selection was based upon available toxicity data and upon information gained from a pilot study with nonpregnant rats. The design included 2 trials with 12 mated female rats per group per trial. It was anticipated that for Experiment I (teratology) the data from both trials might be combined. However, for Trial II the 25-mg/m^3 exposure concentration was reduced to 10 mg/m^3 in response to severe toxicity seen at 25 mg/m^3. Also, two groups (six dams/group) pair-fed to the 10- and 25-mg/m^3 groups were added to Experiment I (teratology), and two groups (six dams/group) were added to Experiment II (dams allowed to litter).

The test groups were exposed (whole body) to APFO in 150-liter glass and stainless-steel chambers within which the rats were housed individually in wire-mesh modules. The location of breeding lots in each chamber was rotated daily. Control rats were exposed to in-house air in the same type of chamber for the same duration of gestation. The temperatures of each chamber were recorded hourly each day during the exposure period.

After each exposure, the rats were housed in suspended, wire-mesh cages (two females/cage), and the racks holding these cages were placed in a walk-in hood.

For the gavage portion of this study (Table 1), the 100-mg/kg/day dosage level was judged to be the maximum that the dams could tolerate based upon a preliminary study with pregnant rats.

For both routes of administration the females were mated on an as-needed basis. The day on which spermatozoa were detected in the vaginal lavage, following overnight cohabitation, was designated as Day 1 of gestation (Day 1G). After the necessary number of females were bred, they were ranked within breeding days by body weight and assigned to groups by rotation in order of rank. For Experiment I (teratology), the dams were weighed on the day of arrival, before breeding, and on Days 1, 6, 9, 13, 16, and 21G. They were observed for abnormal clinical signs and changes in demeanor upon arrival at Haskell Laboratory, at breeding, and daily from Days 6 through 21G. Feed consumption was measured during gestation, but for the inhalation route the dams were housed two per cage due to space restriction. To limit possible bias in the examination of maternal and fetal specimens, the dams were coded (group designation unknown to examiner) from just before sacrifice until all maternal and fetal data were collected and until all structural alterations noted among the fetuses were classified.

After sacrifice of the dams by cervical dislocation on Day 21G, abnormalities were identified macroscopically, liver weights were recorded, and the reproductive status of each animal was determined. The number of corpora lutea and implantation sites were counted, and the number and position of all live, dead, and resorbed fetuses were recorded. The uterus of each apparently "non-pregnant" rat was stained with ammonium sulfide (Salewski, 1964) to detect very early resorptions; data collected were used only to determine the incidence of pregnancy. The weight of the intact and empty uterus for each dam was recorded to allow calculation of actual maternal gain in body weight. All live and dead fetuses were weighed and sexed externally and internally, and the live fetuses were examined at a magnification of 2.5× (Ednalite) for external alterations. The Ednalite also was used to count the corpora lutea. About one-half of the fetuses of each litter that were alive when removed from the dam were examined for visceral alterations (Staples, 1974); in addition, all stunted or malformed fetuses were examined similarly.

For the inhalation route, the heads of all fetuses examined for visceral alterations were fixed in Bouin's fluid to permit examination as described by Barrow and Taylor (1969), but only those from the 25 mg/m^3 and control groups (Trial I) were sectioned free-hand and examined under a stereoscope. This included examination of a vertical cross section through the center of the eyes. Sections containing the eyes of three fetuses from each litter of the 25-mg/m^3 group and of two fetuses from each litter of the control group were processed for examination by light microscopy. In Trial II, one fetal head from each of four litters from the 10-mg/m^3 group and the control group were examined under a stereoscope. The eyes were left intact to minimize processing artifacts. The slices containing the intact eyes were processed and examined by light microscopy. In addition, the heads from all fetuses in the group pair-fed to the 25-mg/m^3 group were processed by the method described for Trial I. For the gavage route, the heads of all fetuses examined for visceral alterations and sufficient of the remainder to total two-thirds of each litter were fixed in Bouin's fluid. Examination of two of the fixed fetal heads of each litter included slicing through the center of each eye in vertical cross section. The heads of three additional fetuses from each litter were not cut through the eyes before being processed to permit examination by light microscopy. All histologic specimens were coded for examination, and particular emphasis was placed upon the structural integrity of the lens.

All fetuses, except for the heads of those that were fixed in Bouin's fluid, were fixed in 70% ethanol, eviscerated (if not done previously), macerated in 1% aqueous KOH solution, and stained with Alizarin Red S to permit examination for skeletal alterations.

For Experiment II (dams allowed to litter), the pro-

[9] Atropisol, NDC 0058-0705-15, Cooper Laboratories (P.R.), Inc., San German, Puerto Rico 00753.

[10] James M. Clinton, VMD, 300 Brookmead Drive, Cherry Hill, N.J. 08034.

TABLE 1

EXPERIMENTAL DESIGN

Route		Exposure levels (mg/m^3)	Number mated females/group: Experiment I (teratology)[a]	Experiment II (dams allowed to litter)[b]
Inhalation[c]	Trial I	0	12	12
		0.1	12	12
		1	12	12
		25	12	12
Inhalation[c]	Trial II	0	12	6
		0.1	12	
		1	12	
		10	15	6
		PF10[d]	6	
		PF25[e]	6	
Gavage[f]		0	25	12
		100 mg/kg	25	12

[a] All Experiment I females sacrificed on Day 21 of gestation.
[b] All Experiment II females were sacrificed on Day 23 postpartum; the offspring were sacrificed on Day 35 postpartum.
[c] 6 hr/day from Days 6 through 15 of gestation.
[d] Control group pair-fed to group exposed to FC-143 at 10 mg/m^3.
[e] Control group pair-fed to group exposed to FC-143 at 25 mg/m^3.
[f] Vehicle used was 5 ml corn oil/kg body wt/day from Days 6 through 15 of gestation.

cedures used until Day 21G were the same as for Experiment I (teratology), except that the dams were weighed on Days 1, 6, and 21G (and twice between Days 9 and 16G, for the gavage portion), feed consumption was not measured, and the identity of each offspring within litters was not retained. At least 2 days before expected parturition, each dam was housed in a 33 × 38-cm polycarbonate cage outfitted with a water bottle, and a wire-mesh lid. The bedding (Bed-O-Cobs; 1/4-in. size) was changed weekly. The date of parturition was noted, and it was termed Day 1PP. The dams were weighed and examined for clinical signs on Days 1, 7, 14, and 22PP. All dams were sacrificed on Day 23PP.

The pups from each dam were counted, weighed, and examined for external alterations toward the end of Day 1PP. Pups with external alterations were marked for subsequent identification. Thereafter, each pup was weighed and inspected for adverse clinical signs on Days 4, 7, 14, and 22PP. Neither standardization of litters nor cross-fostering was practiced. The eyes of the pups from Experiment II were examined by an ophthalmologist between Days 15 and 17PP (inhalation—Trial 1) or between Days 27 and 31PP (gavage). All pups were sacrificed on Day 35PP.

The litter was used as the experimental unit for the purpose of statistical evaluation (Staples and Haseman, 1974; Haseman and Hogan, 1975). The significance of differences in the incidence of pregnancy, clinical signs, and maternal death was determined by use of Fisher's exact probability test (Siegel, 1956). A two-way analysis of variance was used to detect differences in feed consumption among breeding lots and between groups. Dunnett's test (Steel and Torrie, 1960) was used to test the statistical significance of differences between the control and APFO groups in maternal body weight, in body weight gain, and in feed consumption when the one-way analysis of variance was significant. The presence of concentration-related responses for the inhalation portion was determined by Jonckheere's test (Jonckheere, 1954). The significance of differences in incidence of structural alterations between the control group and the APFO group was determined by application of the Mann–Whitney U test (Mann and Whitney, 1947). When more than 75% ties occurred in the data, the Fisher's exact probability test was applied (Haseman and Hoel, 1974). The level of significance selected was $p \leq 0.05$. Variability about means was expressed as standard error of the mean (SE) unless stated otherwise. In addition, several reproductive indices were calculated

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for some results from Experiment II (dams allowed to litter).

RESULTS

Inhalation Route

The exposure levels of APFO achieved (±SD), as measured gravimetrically, for nominal exposure concentrations of 0, 0.1, 1, 10, and 25 mg/m^3 were 0, 0.13 ± 0.020, 1.1 ± 0.16, 10 ± 5.4, and 21 ± 9.7 mg/m^3, respectively. Spectrophotometric data were virtually identical. For both trials (Table 2) between 77 and 90% of the atmospheric particulate was ≤10 m; aerodynamic mass median diameters ranged from 1.4 to 3.4 μm. The average mean daily temperatures in the chambers were between 24.5 and 25°C, and individual temperatures recorded ranged between 22.5 and 26°C.

Experiment I (teratology). Clinical signs that were concentration-related appeared only in the dams of the 10- and 25-mg/m^3 groups. Most of the dams developed wet abdomens, which began in the perineal area, had chromodacryorrhea and chromorhinorrhea, and were unkempt. In addition, four of the dams in the 25-mg/m^3 group that survived to Day 21G became very lethargic toward the end of the exposure period. No adverse clinical signs were noted among the dams of the pair-fed control groups.

Feed consumption of the dams in the 10- and 25-mg/m^3 groups from Days 6 through 15G was significantly less than that for the control group (21.8 ± 0.46 vs 23.4 ± 0.38 g, respectively); no significant differences existed between the consumption of the pair-fed groups and the APFO exposed groups to which they were matched. Similarly, the body weight gain of the dams in the 25-mg/m^3 group from Days 6 through 15G was significantly less than that for the control group, but the difference for the 10-mg/m^3 group was not statistically significant (Table 3).

On Day 21G, actual liver weight of the dams in the 25-mg/m^3 group was significantly increased above the control value (Table 3). The liver weights of the control groups that were pair-fed to the 10- and 25-mg/m^3 groups were significantly less than those for the APFO groups to which they were paired, and than that for the control group (Table 3). On a relative weight basis (using corrected Day 21G maternal body weights), the liver weights ($\bar{x}$ ± SE) for the groups exposed to APFO at 10 and 25 mg/m^3 (5.42 ± 0.125, and 6.46 ± 0.222, respectively) were still significantly larger (Mann–Whitney *U* test, two tailed) than that for their respective pair-fed control groups (4.58 ± 0.164, and 4.62 ± 0.103).

The maintenance of pregnancy and the incidence of resorptions among the surviving dams were not adversely affected by exposure to APFO at concentrations up to and including 25 mg/m^3 (Table 3). The mean fetal body weight in the 25-mg/m^3 group was significantly ($p = 0.002$) decreased (Table 3); but, this also was the case ($p = 0.001$) for the control group pair-fed to the 25-mg/m^3 group. The mean

TABLE 2

INHALATION ROUTE: PARTICLE SIZE DATA FOR APFO AERODYNAMIC DUST IN EXPOSURE CHAMBERS

	Exposure day	Mass median diameter (μm)	Geometric standard deviation	% respirable particles (<10 μm)
Trial I	1	1.4	4.5	90
	10	2.8	6.0	88
Trial II	7	3.4	4.3	77

TABLE 3

REPRODUCTION AND FETAL DEVELOPMENT IN RATS EXPOSED TO APFO BY INHALATION OR BY GAVAGE FROM DAYS 6 THROUGH 15 OF GESTATION

Experiment I (teratology)	Inhalation[a] (mg/m^3)					Pair fed[b]		Gavage (mg/kg body wt)	
	0	0.1	1	10	25	10	25	0	100
Females									
No. pregnant[c]/No. mated	23/24	24/24	23/24	15/15	8/12	6/6	5/6	25/25	22/25
No. deaths	0	0	0	0	3[d]	0	0	0	3[e]
No. litters	23	24	23	15	7	6	5	24	22
Mean No. corpora lutea	14.5 ± 0.47[f]	14.3 ± 0.51	15.6 ± 0.58	15.2 ± 0.65	15.1 ± 1.42	13.8 ± 0.48	14.4 ± 1.29	16.1 ± 0.55	16.7 ± 0.87
Mean No. implants	14.0 ± 0.51	13.4 ± 0.41	13.8 ± 0.28	14.2 ± 0.22	14.0 ± 0.62	14.0 ± 0.63	14.0 ± 1.10	13.6 ± 0.57	13.8 ± 0.64
Mean liver weight (g)[g,h]	15.2 ± 0.30	15.0 ± 0.30	15.4 ± 0.35	16.1 ± 0.50	18.0 ± 0.78[i]	12.8 ± 0.50[l]	12.7 ± 0.48[i]	15.4 ± 0.34	16.2 ± 0.44
Mean maternal weight gain (g)[h]									
Days 6–15	57.6 ± 1.69	57.6 ± 2.06	56.7 ± 1.95	50.9 ± 2.21	36.4 ± 5.33[j]	34.9 ± 5.31[j]	22.7 ± 6.93[j]	56.7 ± 2.34	38.3 ± 2.89[j]
Days 16–21	71.4 ± 2.19	68.9 ± 1.92	72.8 ± 1.97	72.9 ± 2.77	68.6 ± 3.41	71.3 ± 4.72	68.9 ± 2.73	72.6 ± 1.31	84.5 ± 3.00[j]
Days 6–21C[k]	56.7 ± 1.90	56.9 ± 2.02	56.4 ± 2.11	49.1 ± 2.52	37.4 ± 5.39[j]	34.3 ± 2.98[j]	27.0 ± 6.07[j]	57.8 ± 2.35	49.8 ± 2.75[j]
Fetal death									
Mean % resorptions per dam	6.6 ± 1.02	5.3 ± 1.34	3.8 ± 0.87[l]	5.2 ± 1.47	5.9 ± 3.50	4.1 ± 2.94	5.3 ± 3.54	7.9 ± 3.94	4.7 ± 1.30
Fetuses									
No. live	299	305	305	202	92	80	66	322	292
Mean No. live	13.0 ± 0.47	12.7 ± 0.39	13.3 ± 0.28	13.5 ± 0.34	13.1 ± 0.70	13.3 ± 0.21	13.2 ± 0.97	13.4 ± 0.32	13.3 ± 0.67
No. stunted	1	0	0	0	1	0	0	0	0
Mean weight (g)[h,m]	4.0 ± 0.04	3.9 ± 0.05	3.9 ± 0.04	3.9 ± 0.07	3.6 ± 0.12[j]	3.9 ± 0.12	3.5 ± 0.09[j]	3.8 ± 0.08	4.0 ± 0.05

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[a] Nominal concentrations of FC-143.
[b] On each day of gestation, nonexposed dams were given the amount of feed consumed on the same gestation day by selected rats in the corresponding exposure group; if the exposed rats were housed two per cage, then their average consumption for each day was the amount offered to the pair-fed rat.
[c] All females in numerator had visible sign of pregnancy evident at autopsy except for one in the 25-mg/m^3 group in which implantations were detected only by ammonium sulfide staining; data from this female were excluded from all other calculations.
[d] Died on Days 12, 13, or 17 of gestation; the first was not necropsied, but the other two had resorptions *in utero*.
[e] One died on Day 11, and two more on Day 12 of gestation; not recorded whether pregnant.
[f] $\bar{x} \pm$ SE.
[g] Nonpregnant animals were excluded.
[h] Significant dose-related response detected by Jonckherre's test ($p < 0.05$).
[i] Significantly different from control value (two-tailed Mann–Whitney U test, $p < 0.05$).
[j] Significantly different from control value by Dunnett's test ($p < 0.05$).
[k] Day 21C body weight denotes the body weight of females excluding the products of conception (i.e., Day 21 corrected body weight).
[l] Significantly different from control value (one-tailed Mann–Whitney U test, $p \leq 0.05$).
[m] Mean fetal weight/litter; stunted fetuses were excluded.

weight of the fetuses in the 10-mg/m^3 group, and in its pair-fed control group, were not significantly different from the control group ($p \geqslant 0.23$). Neither coded stereoscopic and light microscopic examination of fetal eyes from heads that were fixed in Bouin's fluid, nor detailed examination of the remainder of the fetuses, revealed a concentration-related increase in the incidence of fetuses with malformations or variations (Table 4). In the control group that was pair-fed to the 25-mg/m^3 group, the incidence of fetuses with partially ossified sternebrae was significantly increased ($p = 0.04$ by the two-tailed Mann–Whitney U test), as was the incidence of those with variations regarded as being due to retarded development ($p = 0.02$) vs the control value. In the 25-mg/m^3 group the incidence of fetuses with partially ossified sternebrae also was increased, but the difference from the control value was statistically significant only if the one-tailed Mann–Whitney U test was employed.

Experiment II (dams allowed to litter). During the prenatal period, clinical signs that were concentration related appeared only in the 10- and 25-mg/m^3 groups; they were similar in type and incidence to those seen in Experiment I. In the prenatal period maternal body weight gain of the 25-mg/m^3 group was less than that for the control group (Table 5), but the difference was not statistically significant ($p \geqslant 0.05$). No adverse concentration-related effect on reproductive performance was demonstrated among the dams that survived to term, but the weight of the neonates from the 25-mg/m^3 group was significantly less ($p = 0.02$) than the control value (Table 5). By Day 4PP, the difference was no longer statistically significant. Coded external examination of all of the postpartum pups in Experiment II—Trials I and II, and ophthalmoscopic examination of the eyes of those in Experiment II—Trial I did not reveal concentration-related alterations. No further eye examinations were conducted in view of these negative results and those for Experiment I (teratology), which included light microscopic examination of fe-

TABLE 4

FETAL ALTERATIONS IN RATS EXPOSED TO APFO BY INHALATION OR BY GAVAGE FROM DAYS 6 THROUGH 15 OF GESTATION

Experiment I (teratology)	Inhalation[a] (mg/m^3)					Pair fed[b]		Gavage (mg/kg body wt)	
	0	0.1	1	10	25	10	25	0	100
No. examined (fetuses/litters)									
External	299/23	305/24	305/23	202/15	92/7	80/6	66/5	322/24	292/22
Visceral	159/23	161/24	161/23	110/15	51/7	42/6	34/5	171/24	155/22
Head and eyes	90/17	1/1	—[c]	19/6	51/7	—[c]	34/5	221/24	198/22
Skeleton	299/23	305/24	305/23	202/15	92/7	80/6	66/5	322/24	292/22
Total with variations[d]	144/22	140/24	123/23	87/15	55/6	34/6	42/5	154/23	145/21
Avg. % fetuses with variations/litter (±SE)	48.7 ± 5.07	49.4 ± 4.42	40.1 ± 3.43	42.8 ± 5.14	58.4 ± 5.98	42.2 ± 5.91	64.5 ± 10.42	46.5 ± 5.18	48.5 ± 4.35
Total with malformations	2/2	3/3	1/1	1/1	—[e]	—[e]	1/1	2/1	—[e]
Avg. % malformed fetuses/litter (±SE)	0.6 ± 0.44	1.0 ± 0.53	0.4 ± 0.40	0.4 ± 0.41			1.4 ± 1.42	0.7 ± 0.70	

[a] Nominal concentrations of FC-143.

[b] On each day of gestation, nonexposed dams were given the amount of feed consumed on the same gestation day by selected rats in the corresponding exposure group; if the exposed rats were housed two per cage, then their average consumption for each day was the amount offered to the pair-fed rat.

[c] No fetuses examined.

[d] Does not include variations present in malformed fetuses, if present.

[e] No malformed fetuses detected.

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tal eyes in the group exposed to APFO at 25 mg/m^3.

Gavage Route

Five of the 14 suspensions of APFO in corn oil prepared during the study were analyzed. Calculations based upon fluoride ion measurement indicated that the APFO content of individual suspensions ranged from 2.04 to 3.14%; an APFO content of 2.13% was expected.

Experiment I (teratology). Of the 25 dams, 3 given APFO died as opposed to 0 of the 25 dams given only corn oil (Table 3). Those that died had the clinical signs described earlier under Inhalation. During the dosing period the APFO group consumed significantly less feed than the control group (17.2 ± 0.37 vs 21.9 ± 0.48 g, respectively), and gained about one-third less body weight ($p \leq 0.05$). Those that survived to Day 21G remained pregnant, the incidence of resorptions was not adversely affected, and mean fetal weight was not significantly different between the two groups (Table 3). No malformations were detected among the fetuses of the dams given APFO by gavage and the overall incidence of variations was not significantly different from the control value (Table 4). Neither malformations nor variations were revealed by stereoscopic examination of the bisected eyes from two fetal heads per litter (Bouin's fixed) from each group, or by light microscopic examination of the eyes of an additional three fetuses per litter per group. Microscopically, lesions of the fetal lens were not observed.

Experiment II (dams allowed to litter). Again, three of the dams in the APFO group died, but the reproductive criteria studied were not adversely affected among the dams that survived to term (Table 5). Neither external examination of the neonates nor *in vivo* examination of their eyes between Days 27 and 31PP demonstrated adverse effects related to APFO administration.

DISCUSSION

APFO-related teratogenicity was not demonstrated in this study after administration of the fluoropolymer to rats, by gavage or by inhalation, throughout the period of major organogenesis even at exposure levels that included those lethal to some of the dams. Embryo or fetal toxicity, expressed as decreased fetal weight, was demonstrated, but only after inhalation of APFO at 25 mg/m^3 which was the highest exposure level tested. This probably was due to decreased maternal feed consumption rather than to a direct response to APFO, since the fetuses of the control group pair-fed to the 25-mg/m^3 group also were significantly smaller than the control fetuses. Results from additional APFO exposed dams revealed that the significant difference was temporary, since it did not persist to Day 4PP.

The types of lens changes previously reported to the EPA by the manufacturer of APFO were detected in several fetuses. However, they were determined not to be related to APFO administration because they occurred at similar incidences among all groups including the control group. Lens clefts were determined to be postmortem artifacts that were caused by cutting through the center of the eyes of Bouin's fixed coronal sections. When the fetal eyes were bisected, the fetal nucleus of the lens (which is normally shifted anteriorally at this stage of gestation) was torn from its loose attachment to the anterior lens capsule and a void (cleft) was formed. The cleft was surrounded anteriorally by the lens capsule, laterally by lens' sutures, and posteriorally by the anterior surface of the fetal nucleus. This artifact was essentially eliminated by processing Bouin's fixed fetal heads that were trimmed on either side of the orbit instead of through the center of the eye.

Examination of the eyes of offspring using focal illumination, indirect ophthalmoscopy, and, when indicated, slitlamp microscopy also did not detect APFO-related alterations.

Therefore, the results obtained in this study did not confirm the teratogenicity of APFO

TABLE 5

REPRODUCTION AND DEVELOPMENT OF OFFSPRING OF RATS EXPOSED TO APFO BY INHALATION OR BY GAVAGE FROM DAYS 6 THROUGH 15 OF GESTATION

Experiment II (dams allowed to litter)	Inhalation[a] (mg/m^3)					Gavage (mg/kg body wt)	
	0	0.1	1	10	25	0	100
Females							
No. pregnant/No. mated	18/18	10/12	11/12	6/6	9[b]/12	12/12	9[c]/12
No. deaths	—[d]				2		3
No. litters	18	10	11	6	9	12	9
Mean maternal weight gain (g)							
Days 6–21G[e]	112 ± 4.3[f]	116 ± 8.1	116 ± 7.8	119 ± 3.3	97 ± 8.8	129 ± 4.2	117 ± 5.0
Days 1PP–22PP[g]	−3.4 ± 0.58	−2.8 ± 0.75	−2.0 ± 0.81	−3.1 ± 0.78	−2.2 ± 0.52	9.6 ± 4.28	4.6 ± 8.73
Offspring							
At delivery (Day 1PP)							
No. live pups/litter	12.3 ± 0.60	12.1 ± 0.80	11.2 ± 1.27	13.3 ± 0.67	11.0 ± 1.26	12.8 ± 0.60	12.8 ± 0.55
No. dead pups (%)	1 (0.4)	1 (0.8)	2 (1.6)		1 (1.0)		1 (1.0)
After delivery (to Day 22PP)							
No. dead	1	1[h]	0	0	0	0	1
No. cannibalized	0	2	0	0	2	1	1
No. live	221[i]	118	123	80	97	152	114
No. males/females	115/106	62/56	70/53	44/36	45/52	76/76	48/66
Viability index (%)[j]	100	99.2	100	100	99.2	100	99.2
Lactation index (%)[k]	99.5	98.3	100	100	99.1	99.5	99.2
Mean weight (g)							

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Day 1PP[l]	6.8 ± 0.11	7.0 ± 0.18	6.7 ± 0.18	6.6 ± 0.23	6.1 ± 0.15[m]	6.9 ± 0.12	6.8 ± 0.17
Day 4PP	10.3 ± 0.25	10.9 ± 0.32	10.9 ± 0.39	9.9 ± 0.43	9.7 ± 0.33	10.4 ± 0.30	10.3 ± 0.34
Day 22PP	50.1 ± 1.82	51.4 ± 1.73	52.0 ± 2.46	48.4 ± 2.15	49.0 ± 0.88	49.5 ± 1.68	49.5 ± 2.15
Alterations							
No. examined externally	222/18[n]	121/10	123/11	80/6	99/9	152/12	114/9
No. malformed		1/1[h]					
No. eyes (pairs) examined *in vivo*[o]	141/12	118/10	123/11		97/9	152/12	114/9
No. with alterations	3/3[p]	3/2[q]				1[r]	1[s]

[a] Nominal concentrations of FC-143.
[b] Does not include two females that did not survive the exposure period, but they had implants *in utero* at necropsy.
[c] Pregnancy status not determined for the three females that did not survive to scheduled sacrifice.
[d] Blanks indicate zero incidence.
[e] G = gestation.
[f] Mean ± SE.
[g] PP = postpartum.
[h] Pup with abnormal gait and domed head sacrificed on Day 20PP; it was hydrocephalic.
[i] Pup sacrificed as control for pup from 0.1-mg/m^3 group.
[j] % of pups born that survived to Day 4PP or longer.
[k] % of pups alive on Day 4PP that survived to Day 22PP.
[l] Significant exposure-related response detected by Jonckheere's test ($p \leq 0.05$) for the inhalation route.
[m] Significantly different from control value (two-tailed Mann–Whitney U test, $p \leq 0.05$).
[n] Pups/litters.
[o] On Days 15–17PP examined the eyes of all live pups from Experiment II, Trial I, and on Days 27–31PP all those of live pups from females exposed by gavage.
[p] Opacities of posterior lens pole.
[q] Two with preretinal hemorrhage and one with incomplete mydriasis and red oval opacity on the central endothelium.
[r] One male pup had a band of focal retinal degeneration ventral to the disc in the right eye.
[s] One female pup had corneal edema with superficial vascularization present in the temporal quadrant of the left eye.

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in the rat as previously reported to the U.S. Environmental Protection Agency (EPA) under Section 8(e) of the Toxic Substances Control Act (TSCA).[3] On the basis of the results of this study and additional studies conducted by Du Pont and the manufacturer of APFO, female employees were permitted to return to their original workplace on a voluntary basis.

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